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Differential Salicylate–Aspirin Interaction on Vascular Prostacyclin and Platelet Thromboxane Synthesis in Patients Undergoing Saphenectomy (42213)

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Abstract. We studied the ability of salicylate to counteract the effect of aspirin on platelet thromboxane synthesis and prostacyclin formation in venous tissue in patients undergoing saphenectomy. A single intravenous dose of 40 mg aspirin completely blocked thromboxane formation and reduced prostacyclin to about 43% of the control values. When salicylate (1000 mg po) corresponding in anesthetized subjects to blood levels of 25.9 ± 5 μg/ml was administered before aspirin, vascular prostacyclin was no longer inhibited, whereas platelet thromboxane was still significantly blocked. These results suggest that the combination of salicylate with aspirin at an appropriate dose ratio may result in almost complete dissociation of the drug's effect on platelets and vessels in man.

Although the antithrombotic action of aspirin has been widely studied, considerable uncertainty still exists about the optimal dosage regimen in the management of thrombosis prevention (1). The use of aspirin as antithrombotic agent is based on its inhibitory activity on thromboxane A_2 (TxA_2) in platelets (2, 3). However, the drug also inhibits prostacyclin (PGI_2) in the vessel wall thus possibly reducing its antithrombotic potential (4). Much effort has been dedicated to finding the most appropriate treatment schedule with aspirin to dissociate the drug's platelet and vascular effects.

Salicylate (SA), inactive by itself as an inhibitor of prostaglandin synthesis, has been reported *in vitro* and in animal studies to prevent the inhibitory effect of aspirin on platelet and vascular cyclooxygenase. The possible mechanism of salicylate–aspirin interaction on platelet and vascular cyclooxygenase has been previously discussed by several investigators and appears to involve a competitive inhibition at specific cyclooxygenase binding sites [see for a brief review, Ref (5)]. We reported that in the rat SA was more effective in inhibiting aspirin activity on vascular than on platelet cyclooxygenase (6). We found that at a salicylate–aspirin dose ratio of 10:1 platelet TxA_2 was almost completely inhibited leaving 80–90% of PGI_2 activity at vascular level. In a recent study on healthy volunteers we showed that salicylate at appropriate dose ratio prevented the inhibitory action of aspirin on platelets (7).

The aim of this study was to establish whether salicylate and aspirin also interact at vascular level in man and whether this interaction is apparent at salicylate levels lower than those able to interfere in platelets. The results suggest that the combination of salicylate with aspirin at an appropriate dose ratio leads to almost complete dissociation of the drug's platelet and vascular effects.

Materials and Methods. *Subjects.* Twenty-one female patients admitted to the Milan University Institute of Vascular Surgery for removal of varicose veins were studied. Any history of aspirin sensitivity or prior bleeding excluded a patient from participation. The patients selected for this study were assigned to one of the three treatment groups on the basis of the admission sequence.

Patients who smoked, with previous history of diabetes, hypertension, or myocardial infarction or taking nitrates, beta blockers, or

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calcium channel blocking agents were also excluded from the study. The group of patients who received aspirin or the combination of aspirin and salicylate did not differ significantly from the untreated group with respect to age (39.7 ± 3 years for controls compared to 40.7 ± 7 for aspirin-treated group and 42.3 ± 3 for aspirin–salicylate group, means ± SEM), platelet count (2.51 ± 0.3 × 10^{11}/liter in controls compared to 2.52 ± 0.2 × 10^{11}/liter and 2.34 ± 0.2 × 10^{11}/liter for aspirin and aspirin–salicylate groups, respectively) and hematocrit (44.1 ± 4% in controls compared to 40.3 ± 1% and 39.6 ± 3% for aspirin and aspirin–salicylate groups, respectively). All patients gave their informed consent to the treatment. The research was carried out according to the principles of the declaration of Helsinki and approved by the Human Experimental Committee at "Mario Negri" Institute.

Seven patients were asked to take no medications preoperatively, six patients were given aspirin (Flectadol, Maggioni, Milan, Italy) 40 mg iv 1 hr before removal of the varicose veins. Eight patients took SA (1000 mg po Farmitalia Carlo Erba, Milan, Italy) 1 hr before aspirin. In this last group just before aspirin administration, 5 ml venous blood was taken from an antecubital vein on 3.8% sodium citrate (9:1 v/v) for measurement of SA blood levels. During surgery, segments of the superficial epigastric vein in close proximity to the saphenous vein were removed and immediately placed in sterile saline solution. Gross examination of the vessel segments showed no signs of varicose alteration. At the same time 5 ml of venous blood were taken on sodium citrate.

Vessels and blood samples were transferred to the laboratory for measurement of PGI_2, TxA_2 production, and SA levels as described below.

Vessel processing and testing. The vessel segments (about 2 mm long) dissected free of adventitious tissue were incubated in 100 μl of Tris–HCl buffer (pH 8.9) with arachidonic acid (Nu Check Prep. Elysian, Minn.) (50 μ*M*) for 5 min at 37°C to stimulate PGI_2 production. The vessels were then removed, weighed, and the buffer was frozen at −20°C for measurement of PGI_2 by a radioimmunoassay of its stable metabolite 6-keto-$PFG_{1\alpha}$ as previously described in detail (8). The antibody for 6-keto-$PGF_{1\alpha}$ determinations was a kind gift of Dr. M. J. Buchanan (Mc Master University, Hamilton, Ontario, Canada).

Blood processing. Citrated venous blood was divided into two 1-ml portions. One was mixed with thrombin (5 NIH U/ml, Toposstasine, Roche, Milan, Italy) and the other with the corresponding amount of buffer. The samples were then incubated at 37°C for 30 min. Blood samples were centrifuged at 10,000*g* for 3 min and the supernatant serum or plasma was frozen until assayed for TxA_2. TxA_2 was measured in appropriately diluted serum samples by radioimmunoassay of its stable metabolite TxB_2 as previously described (9). TxB_2 levels before aspirin in serum formed by thrombin clotting of citrated blood were about 10 times lower than those previously measured in serum obtained by spontaneous clotting of native blood (7). SA in blood was measured by a spectrophotometric assay (10).

Results. As shown in Fig. 1 the level of TxB_2 in serum of untreated patients was 44.8 ± 4 pmole/ml and 6-keto-$PGF_{1\alpha}$ produced by the vessels was 2.73 ± 0.2 pmole/mg wet wt. TxB_2 in plasma from blood without addition of thrombin was always below the sensitivity of the method (data not shown). After aspirin administration, TxB_2 generation was completely inhibited in all treated patients and 6-keto-$PGF_{1\alpha}$ generation was reduced to about 43% of the control values.

When salicylate was administered 1 hr before aspirin, TxB_2 generation in serum remained undetectable in three subjects but reached a detectable level (about 10–15% of controls) in five patients. In all but two subjects 6-keto-$PGF_{1\alpha}$ values were within the range for controls. TxB_2 was undetectable in one of the two patients with low 6-keto-$PGF_{1\alpha}$ value. The mean value of 6-keto-$PGF_{1\alpha}$ was reduced by 23% compared to control values but this reduction was not statistically significant. Mean SA blood levels at the time of aspirin administration were 25.9 ± 5 μg/ml (range 7.8–59.5). The two subjects with the lowest values of 6-keto-$PGF_{1\alpha}$ (1.3 and 1.24 pmole/mg tissue) also had the lowest salicylate plasma levels (7.8 and 15.6 μg/ml, respectively).

Discussion. The results of this study show that salicylate *in vivo* in man prevents the in-

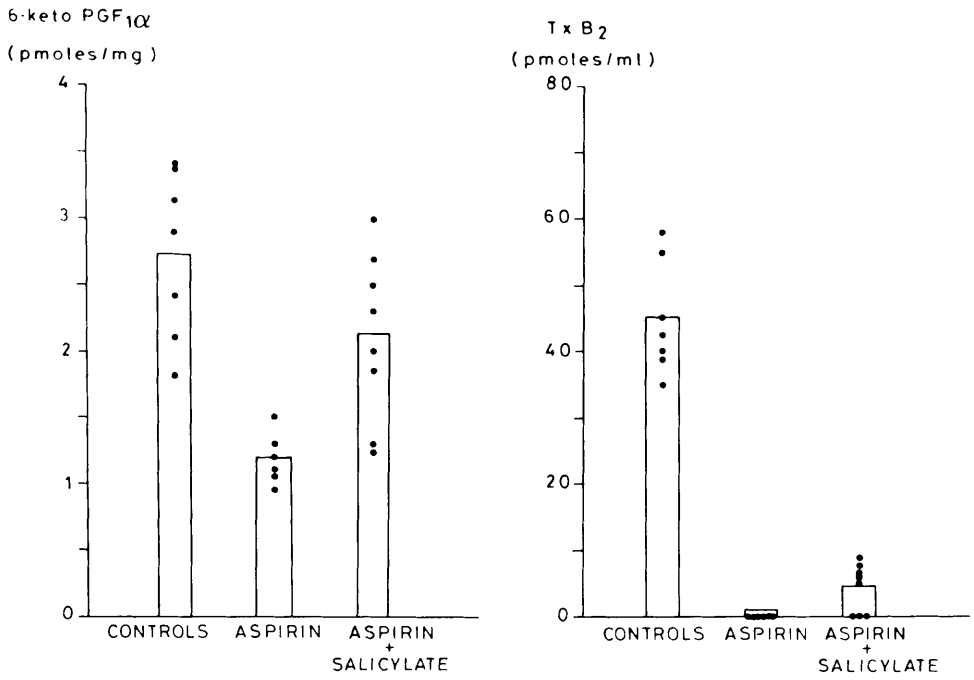

FIG. 1. Inhibition of vascular 6-keto-$PGF_{1\alpha}$ and platelet TxB_2 production by 40 mg iv aspirin and prevention of this inhibition by earlier administration of sodium salicylate (1000 mg po). Seven subjects did not take any drug, six subjects were given aspirin alone, and eight subjects were given aspirin 1 hr after salicylate. Statistical analysis by Duncan's test showed significant differences ($P < 0.01$) between control and aspirin-treated group and between aspirin and aspirin–salicylate group for 6-keto-$PGF_{1\alpha}$ generation. Statistical analysis by Student's t test showed significant differences ($P < 0.01$) between control and aspirin–salicylate group for TxB_2. The three samples in the latter group which were below the detection limit of the assay were considered as measuring the lowest detectable value of TxB_2.

hibitory action of aspirin on vascular cyclooxygenase activity. A single oral dose of salicylate (1000 mg) prevented the inhibitory activity of 40 mg aspirin (iv). Previous studies on human volunteers (7) showed that this was the minimal single iv dose of aspirin preventing platelet TxA_2 production and sodium arachidonate-induced aggregation by more than 90%.

Our results show that after this aspirin dosage TxB_2 values in all subjects fell below the limit of detection and venous prostacyclin production was blocked by about 50%. Similar inhibition of vascular PGI_2 and platelet TxA_2 has been reported by other authors after oral administration of 80–150 mg aspirin (11–13).

When SA was administered before aspirin, TxB_2 remained undetectable in three patients and reached 10–15% of control values in the other five. In contrast six patients generated PGI_2 levels within the control range and only two appeared to be partially inhibited.

When analyzed in respect to the study in healthy male volunteers, the same salicylate–aspirin dose ratio (1000 mg oral versus 40 mg iv) resulted in significant prevention of the aspirin effect on platelet TxB_2 generation in the group of volunteers, but not in patients. The most likely explanation is that at the moment of aspirin administration the plasma levels of salicylate, measured 1 hr after ingestion (25.9 ± 5 μg/ml), were markedly lower than those measured 40 min after ingestion in volunteers (67.3 ± 5.1 μg/ml). In fact salicylate levels in patients were similar to those found in volunteers 40 min after 250 mg salicylate (22.3 ± 1.7 μg/ml). In the latter condition, as well as in patients, no significant prevention of aspirin's effect on TxB_2 synthesis could be de-

tected (7). Possibly, gastrointestinal absorption of salicylate was reduced in patients by preanesthesia (14).

The possibility of sex-related difference in the systemic availability of SA and/or aspirin between male volunteers and female patients is unlikely. Indeed the elimination and systemic availability of both aspirin and SA were equal in two groups of men and women studied by Husted *et al.* (15). Moreover, the inhibition by SA of the antiaggregatory effect of aspirin was also similar in both sexes (15). Thus, we applied to female subjects the same SA–aspirin treatment scheme previously shown to effectively interact on platelets from male subjects. The choice of females for the present study was due to the fact that about 90% of the patients admitted for saphenectomy to our hospital were indeed female.

Independently of the reasons why the same dose of salicylate gave rise to different plasma levels of the drug in the two groups of subjects, it appears that the plasma levels of salicylate rather than the amount of the drug administered are relevant for an optimal interaction with aspirin.

The observations reported on the differential sensitivity of platelet and vascular cells to salicylate inhibition of the aspirin effect confirm in man the results in the rat (6). Using a wide range of aspirin–salicylate doses in the rat we observed that salicylate prevented aspirin's effect on prostaglandins more effectively in vascular cells than in platelets. In the rat, as well as in this study in man, vascular cells showed lower sensitivity to aspirin than platelets. Salicylate administration in both systems resulted in an amplification of the difference in response to aspirin of platelets and vascular cells. The mechanism of this property of SA (i.e., different sensitivity or different access of the drug at various cellular levels) remains to be established.

These preliminary observations, however, may constitute an original approach on which to base better use of aspirin in clinics. Weksler *et al.* (14) reported that low doses of aspirin (80 mg po) largely inhibited platelet aggregation and TxA_2 synthesis with much less effect on arterial and venous PGI_2. It is possible that the use of salicylate in combination with aspirin further augments the differential effects of aspirin on prostaglandins in a supposedly beneficial direction.

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